THE MAIN OUTLINES OF BACTERIAL CLASSIFICATION

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1. INTRODUCTION

"Was diese Wissenschaft betrifft,
Es ist so schwer, den falschen Weg zu meiden,
Es liegt in ihr so viel verborgnes Gift,
Und von der Arzenei ist's kaum zu unterscheiden."
—GOETHE

Although a great deal has been written on bacterial taxonomy during the past few decades, a perusal of the literature shows that for the most part this work has been restricted to the classification of the *Eubacteriales* alone. Since the early days of microbiology, comparatively little attention has been paid to the broader problem of delimiting and defining the *Schizomycetes* as a whole and the major groups contained therein. Nevertheless, it can hardly be contended that this is an unimportant aspect of bacterial taxonomy; on the contrary, a clear recognition of the larger *natural groups* of bacteria, their characteristics and relationships, would seem to be an indispensable basis for more detailed work.

The increased use of Bergey's *Manual of Determinative Bacteriology* for purposes of identification, together with the obvious attempts made by the present Board of Editors to seek collaboration with specialists on various groups, make it likely that this *Manual* will ultimately become the internationally recognized and authoritative handbook on bacterial taxonomy. Nevertheless, in its main outlines the system used in Bergey's *Manual* is still far from satisfactory. There will in due course be a succeeding edition, and it is with the hope of contributing some constructive suggestions for its outline that the present essay is offered.

2. PHYLOGENY AND EMPIRICISM IN BACTERIAL SYSTEMATICS

In most biological fields it is considered a truism to state that the only satisfactory basis for the construction of a rational system of classification is the phylogenetic one. Nevertheless, "realistic" bacteriologists show a curious aversion to the attempted use of phylogeny in bacterial systematics. This is well illustrated, for example, by the statement of Breed (1939):

> Realistic workers have on their side been impatient with idealists who have introduced many . . . unjustified speculations regarding relationships between the various groups of bacteria.

To what may we ascribe this distrust of phylogeny? In part it is undoubtedly due to the unsatisfactory nature of certain systems, purportedly based on phylogeny, which have been proposed in the past. However, the mere fact that a particular phylogenetic scheme has been shown to be unsound by later work is not a valid reason for total rejection of the phylogenetic approach.

Another important reason for the "realistic" attitude is the widespread belief that bacteria present too few characters on which schemes of relationships (and hence a natural system) can be based. It is our belief that such pessimism is not entirely justified, and that at present some relationships can be recognized which can well be incorporated in a system of classification.

Even granting that the true course of evolution can never be known and that any phylogenetic system has to be based to some extent on hypothesis, there is good reason to prefer an admittedly imperfect natural system to a purely empirical one. A phylogenetic system has at least a rational basis, and can be altered and improved as new facts come to light; its very weaknesses will suggest the type of experimental work necessary for improvement. On the other hand, an empirical system is largely unmodifiable because the differential characters employed are arbitrarily chosen and usually cannot be altered to any great extent without disrupting the whole system. Its sole ostensible advantage is its greater immediate practical utility; but if the differential characters used are not mutually exclusive (and such mutual exclusiveness may be difficult to attain when the criteria employed are purely arbitrary) even this advantage disappears. The wide separation of closely related groups caused by the use of arbitrary differential characters naturally enough shocks "idealists," but when these characters make it impossible to tell with certainty in what order a given organism belongs, an empirical system loses its value even for "realists."

It seems unnecessary to give here an exhaustive review of bacterial systematics. The reader is referred to Buchanan's scholarly treatise on general systematic bacteriology for an excellent survey of this field up to 1925. More recent literature has been briefly reviewed by Breed in the latest edition of Bergey's *Manual.*

We shall, therefore, restrict ourselves to a critique of Bergey's system which illustrates well the weaknesses of the empirical approach.

3. A CRITIQUE OF BERGEY'S SYSTEM

Admittedly it is a difficult task to frame a definition of the *Schizomycetes* adequate to include all organisms which belong here but sufficiently specific to exclude other groups of microorganisms. Nevertheless, a more inadequate definition than that given by Bergey would be hard to conceive. Bacterial cells are described as "relatively primitive in organization," but one looks in vain for an explicit statement of the absence of true nuclei, which is perhaps the most important single morphological characteristic of these organisms. In describing cell shape, the word *filamentous* is used in a most confusing manner; apparently it is applied indiscriminately to the usually non-septate mycelium of the actinomycetes, to chains of individual cells such as occur in the *Bacilleae* and to the truly filamentous (i.e. multicellular) arrangement found in the *Beggiatoaceae*. Multiplication is given as being typically by "cell fission," but there is no mention of its wellnigh universal transverse nature. The absence of sexual reproduction is not noted. The formation of endospores and conidia is mentioned but not the formation of myxobacterial spores and cysts. Next comes the astonishing statement, "Chlorophyll is produced by none of the bacteria (with the possible exception of a single genus)." The occurrence of a true chlorophyll—though chemically slightly different from the green plant chlorophylls a and b—in all the purple bacteria, both *Thio-* and *Athiorhodaceae,* invalidates the inclusion of this character. In the description of motility, the peculiar locomotion so characteristic of the *Myxobacteriales* goes unmentioned; the motility of the *Spirochaetales* is described as "flexuous," which certainly does not characterize the mode of locomotion of these forms in any adequate manner.

In order to appreciate the complete inadequacy of this definition of the class *Schizomycetes* one has only to realize that there is nothing in it which would exclude the fungi and most of the protozoa, whereas the statement about the absence of chlorophyll (clearly put in originally to keep out the algae) now also excludes a whole family of the *Thiobacteriales.*

The differentiation of the seven orders used in the *Manual* is no more satisfactory. The first order of the *Eubacteriales* is carefully segregated from the rest as containing "simple and undifferentiated forms." No mention is made of the flagellar nature of motility or the rigidity of the cell wall, which are really important characters in this order. The statement that "iron (is) not stored as visible particles" applies equally well to all other living organisms.

The remaining orders are described as "specialized or differentiated"; in the absence of a definition of these two terms the characterization becomes entirely meaningless. Even if, from a consideration of the organisms thus grouped together, it would seem possible to sense the implication of these terms, it must be pointed out that forms no more "specialized or differentiated" than members of the *Eubacteriales* have been incorporated in these orders. To mention an example: in the second order of the *Actinomycetales* (which is separated from the following orders as being "mold-like") one finds the genera *Mycobacterium* and *Corynebacterium.* It is clearly illogical to describe these genera either as "mold-like" or as "specialized and differentiated," an opinion which is substantiated by the fact that in the fifth edition one can find indubitable *Corynebacterium* species described in no less than three families of the *Eubacteriales* (*Rhizobiaceae*, *Pseudomonadaceae* and *Bacteriaceae*). The third, fourth and fifth orders *Chlamydobacteriales*, *Caulobacteriales* and *Thiobacteriales* are collectively described as "alga-like." Clearly the recognition of the relationship of organisms such as the *Beggiatoaceae*, *Clonothrix*, etc., to representatives of the order *Hormogonales* of the *Myxophyta* has prompted the inclusion of this character in the descriptive diagnosis of these orders. But the term "alga-like" is entirely too general since it implies some unspecified resemblance (such as habit of growth?) to *some* organisms included in *some* of the seven divisions of the algae. Furthermore, this statement applies only to some of the organisms in each order, certainly not to all; in the *Caulobacteriales* one can find organisms morphologically very similar to the *Eubacteriales* except for the possession of a stalk or holdfast (*Nevskia pediculata*, *Caulobacter vibrioides*), while the representatives of the entire family *Rhodobacteriaceae* (*Thiobacteriales*) are morphologically indistinguishable from their colorless counterparts in the *Eubacteriales*.

The artificiality of the *Chlamydobacteriales*, *Caulobacteriales* and *Thiobacteriales* is clearly shown by the fact that the important differential characteristic for each order is also exhibited by species which have been placed in one of the other two orders. Thus, a sheath, which is the key character of the *Chlamydobacteriales*, occurs in the genus *Thioploca* (*Thiobacteriales*); several *Leptothrix* (*Chlamydobacteriales*) and *Thiothrix* (*Thiobacteriales*) species are attached to the substratum by a holdfast (*Caulobacteriales:* "in some species the stalks may be very short or absent, the cells connected directly to the substrate or to each other by holdfasts"); and finally, the species *Nevskia ramosa* (*Caulobacteriales*) shows evidence of containing sulfur globules, which might suggest an alternative position in the *Thiobacteriales*. Even if these species can be placed in the order to which they have been assigned on the basis of other characters, such a situation is apt to cause confusion.

The description of the sixth order *Myxobacteriales* as "slime mold-like" would hardly appeal to anyone familiar with the organisms belonging to the two groups. The absence of true nuclei, of sexual reproduction, and of amoeboid cell form in the *Myxobacteriales* shows quite clearly the fundamental lack of similarity to the *Myxomycetae*. Scientific descriptive keys should not contain such misleading comparisons. On the other hand, the two most important characters of the *Myxobacteriales*, the type of locomotion and the absence of rigid cell walls, are not mentioned in the key.

After this, it is not surprising to find the seventh order *Spirochaetales* differentiated from the rest as "protozoan-like." The further characterization is so diffuse that it gives no helpful information concerning this group of organisms. Certainly the determinative significance of the statement "Some forms transmitted by insect vectors" is not apparent.

The stress laid on these points may seem unnecessary. However, the fact that the definitions and segregations of the various orders have remained unchanged through five consecutive editions of Bergey's *Manual* shows that its weaknesses (not only from the scientific, but even from the determinative standpoint) have not been generally realized.

4. PRINCIPLES FOR THE DETERMINATION OF RELATIONSHIPS

What are the characters on which the recognition and separation of natural groups may be based? In the classification of higher plants and animals, systematists have relied almost exclusively on morphology. Nevertheless, some exceptions to this rule may be found, particularly in the treatment of the thallophytes, where an increasing reliance appears to be placed on physiological characters. For example, Smith (1938) in breaking up the algae into seven new divisions, places as much weight on physiological characters (reserve products, nature of the cell wall, pigments) as on morphological ones.

It is above all in bacterial systematics that extensive use of physiological criteria has been made. This is understandable enough in view of the paucity of morphological data but, as pointed out by Kluyver and van Niel (1936), the injudicious use of physiology without a clear understanding of what constitute important physiological characters, has led to much confusion. A good example of this is the order *Thiobacteriales* of Buchanan, which is based on the presence of "bacteriopurpurin and/or sulfur granules" in the cells. In addition to uniting exceedingly heterogeneous morphological groups, these characters also bring together two radically different physiological groups, the photosynthetic purple bacteria and some of the chemosynthetic colorless sulfur-oxidizing bacteria. Furthermore, extreme physiological systems have often neglected obvious morphological relationships, with the result that natural morphological groups have been split up or forced into assemblages with which they have little in common save certain aspects of metabolism. The *Thiobacteriales* again are a good example of this; other even more glaring ones may be found in the system of Orla-Jensen (1909) where, for example, the genera *Mycobacterium*, *Corynebacterium* and *Actinomyces* are placed among the cephalotrichous bacteria in one family with the genus *Rhizobium*!

The chief stumbling block in attempting to draw up a phylogenetic system on a primarily physiological basis is the necessity of making a large number of highly speculative assumptions as to what constitute primitive and advanced metabolic types. Orla-Jensen, for example, regarded the chemosynthetic bacteria as the most primitive group because they can live in the complete absence of organic matter and hence are independent of other living forms. This overlooks the fact that a chemosynthetic metabolism necessarily presupposes a rather highly specialized synthetic ability such as one would not expect to find in metabolically primitive forms. Furthermore, this reasoning was based at least in part on the hypothesis that living forms arose at a time when the earth was devoid of organic matter, an hypothesis which has been effectively challenged by Oparin (1938) in his book on the origin of life. According to Oparin, it is probable that a long period of chemical synthesis of organic material preceded the emergence of life, and that consequently the earliest living forms were heterotrophs. On this reasoning, the development of autotrophism was a later adaptation to an environment in which organic materials had become scarce through the activities of heterotrophs.

Thus, the basic assumption used by Orla-Jensen in erecting a physiological phylogenetic system has been rendered, to say the least, highly doubtful. The physiological reasoning on which the further development of the system is founded is also open to serious criticisms.

In spite of the comparative simplicity of bacteria it is rather naïve to believe that in the distribution of their metabolic characters one can discern the trend of physiological evolution. For these reasons, a phylogenetic system based solely or largely on physiological grounds seems unsound. It is our belief that the greatest weight in making the major subdivisions in the *Schizomycetes* should be laid on morphological characters, although correlative physiological characters may also be used.

What, then, are the basically important morphological characters which we can use? Clearly paramount is the structure of the individual vegetative cell, including such points as the nature of the cell wall, the presence and location of chromatin material, the functional structures (e.g., of locomotion), the method of cell division, and the shape of the cell. A closely-allied character is the type of organization of cells into larger structures. In addition, the nature and structure of reproductive or resting cells or cell masses deserve due consideration.

In the following sections we will examine the major bacterial groups which we can discern by the application of these criteria, elucidating as far as possible the relationships of these groups to each other and to other microörganisms.

5. A SURVEY OF THE BACTERIA

A. The Eubacteriales and related assemblages

The group which naturally presents itself first is that of the *Eubacteriales*. These organisms always possess rigid cell walls, demonstrable in the larger species by plasmolysis. Motility is by no means universal, but where it does occur, it is always flagellar. Cell division is always by transverse fission. While true nuclei are absent, there have been a number of reports in recent years, based on the use of reliable cytological techniques, in which claims have been made for the presence of discrete masses of chromatin material which could be interpreted as primitive chromosome-like structures (Badian, 1933, Stille, 1937, Piekarski, 1937. The work of Schaede (1940) has, however, cast some doubt on these previous results). The endospore, found in certain groups in the *Eubacteriales*, is highly characteristic, occurring nowhere else among the bacteria. A few members (*Azotobacter* spp.) form cysts. Endospores and cysts are the only two types of resting stages found in the *Eubacteriales*.

On a purely morphological basis one can arrange the representatives of the *Eubacteriales* into a hypothetical tree with four main ascending branches, as was done by Kluyver and van Niel (1936). Certain facts which have come to light since 1936 have tended to weaken parts of this scheme, but in spite of that it is still useful for illustrating broad trends within the group. Also, some of these trends clarify the relationships between the *Eubacteriales* and other assemblages. A slightly modified form of the tree is given in figure 1.

Starting from the hypothetical primitive coccus type, the first line leads through the micrococci to the sarcinae, culminating in the spore-forming sarcinae. These forms may be either gram positive or gram negative. Motility occurs infrequently in members of this group, and spore formation has so far been reported only in *Sporosarcina ureae*.

The second line consists of the polarly flagellated rods, starting with the pseudomonas type and leading through the vibrios to the spirilla. The representatives of this line are all gram negative with one possible exception. This is the species *Listeria* (*Listerella*) *monocytogenes* (Pirie 1940) which was originally described as a gram-positive polarly flagellated rod (Pirie 1927) but which has been more recently reported as peritrichously flagellated (Paterson 1939), and hence perhaps does not belong in this line. Spore formation is rare, being well established for only one species, *Sporovibrio desulfuricans* (Starkey 1938). There have also been reports, so far unsubstantiated, of spore formation in *Spirillum* species (see Starkey 1938, and Lewis 1940).

The third line is morphologically highly diverse. It takes its origin in the streptococci, and passes through the lactic acid rods, the propionic acid and other corynebacteria, and the mycobacteria to the actinomycetes. All members of this line are gram positive and none form endospores. Until very recently motility was thought to be absent in the higher forms although motile streptococci (see Koblmüller, 1935) had been known for some time. However, the work of Topping (1937) has shown that there are organisms of the *Mycobacterium-*

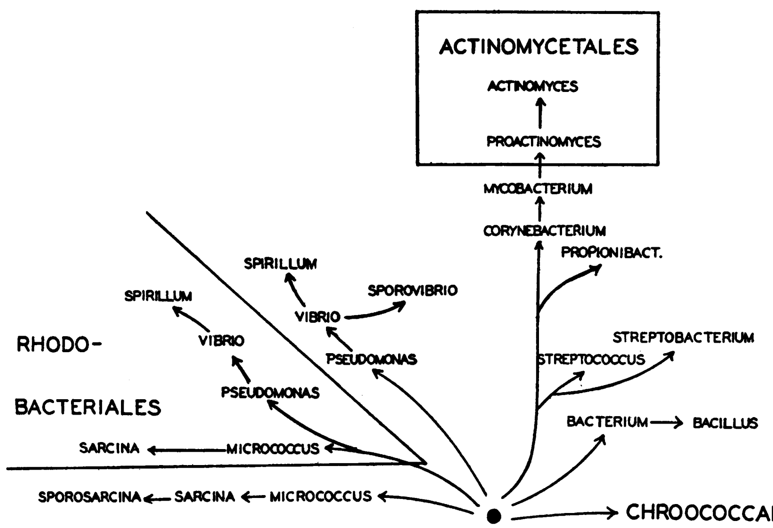

FIG. 1. PHYLOGENY OF THE *Eubacteriales* AND RELATED GROUPS, ACCORDING TO THE SCHEME OF KLUYVER AND VAN NIEL WITH SLIGHT MODIFICATIONS

The names used denote morphological entities, not necessarily genera. For example, the designation "Bacterium" includes all genera of non-sporeforming, peritrichously flagellated rods: *Kurthia, Escherichia, Aerobacter*, etc.

Proactinomyces type which are flagellated. This fact may ultimately necessitate a radical revision of this line since, if motility is conceded to occur all along it, there is no valid means of differentiating between gram-positive peritrichously flagellated rods of the *Kurthia* type (at present placed in the fourth line) and organisms belonging to the genera *Mycobacterium* and *Corynebacterium*.

The fourth line of the Kluyver-van Niel scheme comprises all peritrichously flagellated rods; both gram-positive and gram-negative forms occur. It is here that the great majority of sporeformers have up till now been placed. This line is also an unsatisfactory one because, particularly in the spore-forming representatives, a number of morphologically rather distinct types can be recognized. It is probable that future work will show the necessity of drastic revisions, but on the basis of present knowledge it is difficult to make satisfactory modifications of this and the preceding line.

The above-mentioned four lines were postulated primarily on the basis of cell shape and mode of insertion of flagella. It is of importance to note that the two most satisfactory groups share an additional character, *viz.*, the homogeneous behavior of the members with respect to the gram stain. In the remaining two lines no such uniformity exists at the present time. This makes it necessary to determine the relative values of the type of flagellation and of the gram reaction for systematic purposes. The various recent reports on the difficulties encountered in definitely ascertaining the mode of insertion of flagella (Pijper, 1930, 1931, 1938, 1940; Pietschmann, 1939; Conn, Wolfe and Ford, 1940) lead one to suspect that the gram stain may ultimately appear to be the more valuable.

There are two large groups which show obvious morphological relationships to the *Eubacteriales;* namely, the *Actinomycetales* and the photosynthetic bacteria.

The *Actinomycetales* have already been mentioned in connection with the third line in the *Eubacteriales* from which they are clearly derived. The delimitation of this order is a difficult and necessarily arbitrary matter, since the morphological series which takes its origin in the lactic acid bacteria runs practically unbroken to the most complex of actinomycetes. From the purely determinative standpoint it is probably most satisfactory to start the *Actinomycetales* with the *Proactinomycetaceae*, the group in which a primitive mycelium formation occurs. In this case a clear and simple delimitation of the order is possible. As was mentioned in the critique of Bergey's system, the inclusion of the mycobacteria and corynebacteria leads to confusion, since these forms can so readily be taken for representatives of the

Eubacteriales. On the other hand, the dividing line between the genera *Mycobacterium* and *Proactinomyces* is a tenuous one.

Like the *Eubacteriales*, the *Actinomycetales* always possess rigid cell walls. Schaede (1940) has shown that the distribution of chromatin material in them is identical with that in members of the *Eubacteriales*. The question of motility in this order must remain open for the present, although it seems likely that the motile organisms described by Topping (1937) should be placed here. All representatives of the *Actinomycetales* are gram-positive; for this reason the inclusion here of the polarly flagellated gram-negative genus *Mycoplana*, tentatively suggested by Waksman (1940), is undesirable. Endospores are never formed in the *Actinomycetales*; the characteristic reproductive structures in this order are conidia, formed by fragmentation of the aerial hyphae.

The photosynthetic bacteria were first rationally treated in the system of Pringsheim (1923), who recognized their differences from the colorless sulphur bacteria with which they had so long been associated, and created for them the order *Rhodobacteriales*. Kluyver and van Niel placed them in the *Eubacteriales*, an action which was entirely justified from the strictly morphological standpoint. These organisms are morphologically indistinguishable from the true bacteria, falling into the *Pseudomonas-Vibrio-Spirillum* and *Micrococcus-Sarcina* lines. All species are gram-negative and non-spore-forming. Nothing is known about the distribution of chromatin material. Chromatophores are absent, the photosynthetic pigments being evenly distributed throughout the cell, as in the blue-green algae. Physiologically, these organisms differ from green plants in a number of respects. Bacteriochlorophyll is chemically slightly different from chlorophylls a and b. Photosynthesis is accomplished only in the presence of reducing substances, and never accompanied by oxygen production.

The photosynthetic bacteria form a homogeneous group whose photosynthetic metabolism sets them off from the *Eubacteriales*. For this reason, it seems wise to keep them as a separate order, recognizing nevertheless their close relationship to groups in the true bacteria.

The three assemblages discussed so far—*Eubacteriales*, *Actinomycetales* and *Rhodobacteriales*—are a well-knit, closely related natural group whose relationships to other bacteria and to non-bacterial microörganisms are not very clear.

A number of workers (Drechsler, 1919; Vuillemin, 1912, 1925) have postulated a relationship between the *Actinomycetales* and the *Eumycetae*, but beyond the superficial resemblances in the mycelial nature of growth and the formation of conidia (oidia) by hyphal fragmentation there is little support for this hypothesis. True nuclei do not occur in the actinomycetes, which, as mentioned previously, show a typical eubacterial arrangement of the chromatin. Furthermore, the width of the individual hyphae is always of bacterial dimensions, never approaching that of the true fungi. Negative evidence is the complete absence of sexual reproduction in the actinomycetes. The superficial similarities between molds and actinomycetes are probably to be regarded as an example of convergence.

The only relationship which has been seriously suggested for the *Eubacteriales* is one with the *Myxophyta*. Although the close relationship existing between bacteria and blue-green algae was stressed by 19th century microbiologists such as Cohn, van Tieghem, and Hansgirg (the two former workers treated them as one group), the importance of this concept has been appreciated less in recent times.

The common features of true bacteria and blue-green algae may be summarized as follows:

1. Absence of true nuclei.
2. Absence of sexual reproduction.
3. Absence of plastids.

One major difference, however, is the complete absence of flagellar motility in the *Myxophyta*, whose representatives are either immotile or exhibit creeping motility.

It is among the *Chroococcales*, the most primitive assemblage of the *Myxophyta*, that we find forms closely resembling the *Eubacteriales*. A *Chroococcus* sp., for example, would be indistinguishable from a *Micrococcus* sp. if it were to lose its photosynthetic pigments. The genus *Eucapsis* would be similarly indistinguishable from the genus *Sarcina*. Thus it seems at least possible that the primitive blue-green algae of the *Chroococcus* type have developed from the *Eubacteriales* as a *second photosynthetic line,* at first paralleling morphologically the purple bacteria, but undergoing in the course of time a far more complex morphological evolution which resulted in the development of the two higher orders, the *Hormogonales* and the *Chamaesiphonales*. If this were the case we must assume that the most primitive blue-green algae were non-motile, being derived from a non-motile group in the true bacteria somewhere close to the primitive coccus type, and that the very characteristic creeping motility of the *Myxophyta* developed at some later time during their evolution, probably in one of the branches of the *Chroococcales*. It should be realized that this is a speculative digression, which does not affect the systematic proposals we shall put forward. It is at least certain that morphologically the *Myxophyta* resemble the true bacteria far more closely than they do any of the other algal groups.

B. The Myxobacteria

The second major natural group of the *Schizomycetes* which we can recognize is that of the myxobacteria. These are forms which have been but little studied by bacteriologists, and our knowledge of them is due almost entirely to the work of such cryptogamic botanists as Thaxter and Jahn.

The vegetative myxobacterial cell differs fundamentally from that of the true bacterium. In the first place, it does not have a rigid cell wall (Thaxter, 1892; Jahn, 1924; Krzemieniewski, 1928); instead there appears to be only a delicate plasma membrane which gives the cell a power of flexibility never found in the true bacteria. Secondly, flagellar motility is completely absent in the myxobacteria, which exhibit instead a creeping motion very similar to that found in blue-green algae. All species studied so far are motile. Division is always by transverse fission. In their staining reactions the myxobacteria differ markedly from the true bacteria; with ordinary aniline dyes the cells are for the most part rather faintly stained, but certain granules within the cells become intensely colored. These granules have been interpreted (Badian 1930) as consisting of chromatin material which acts in a manner similar to that of chromosomes; more work is needed, however, to substantiate these claims. The vegetative cells are always rod-shaped, two types being found: cells of even width with blunt ends, and pointed spindle-shaped cells.

The life cycle of the typical myxobacterium is divided into a *swarm stage* in which the vegetative cells creep across the substrate in loose masses held together by a slimy material, and a *fruiting stage* in which the vegetative cells aggregate and pile up at certain points on the substrate to form fruiting bodies which often are of considerable structural complexity. On or in the fruiting bodies resting cells are borne. In the family *Myxococcaceae* these are spherical, highly refractive cells, each of which is formed by the rounding up and shortening of a single vegetative cell. In the remaining families the resting cells are merely shortened vegetative cells.[1]

Under favorable conditions the resting cells germinate and give rise anew to vegetative cells.

The supposed difficulties of obtaining pure cultures have caused the study of the myxobacteria to be confined largely to the fruiting stage. Hence myxobacteria in which the fruiting stage is absent have not been recognized until recent years. These forms, the cytophagas, possess typically myxobacterial vegetative cells (Stanier 1940); some exist only in the vegetative stage, whereas others form microcysts in a manner similar to that of the *Myxococcaceae*, with the sole difference that organized fruiting bodies are not produced (Krzemieniewska 1930, 1933; Imsenecki and Solntzeva, 1936).

In the myxobacteria we once again find a well-defined, close-knit group whose relationships are doubtful. It is at least certain

[1] These bodies have been called spores, but their mode of formation indicates that a more correct designation would be *cyst*. Since in many genera a large number of these resting cells may be enclosed within a larger envelope which has been called a cyst, the term *microcyst*, suggested by Krzemieniewska (1933) for the individual resting cells, is more satisfactory.

that they are only remotely, if at all, related to the *Eubacteriales-Actinomycetales-Rhodobacteriales* assemblage. Some workers (Thaxter 1904; Vahle 1909) have suggested a relationship with the *Myxomycetae*, particularly with the *Acrasieae*, in which group there also occurs a swarm stage (pseudoplasmodium) consisting of individual vegetative cells. However, as in the case of the putative *Actinomycetales-Eumycetae* relationship, this is based largely on superficial resemblances in the manner of growth. All the *Acrasiae* have definitely amoeboid vegetative cells with true nuclei.

It seems more likely (Jahn 1924, Stanier 1940) that the myxobacteria have developed through forms such as the cytophagas from rod-shaped, motile types among the lower blue-green algae (*Chroococcales*) by loss of chlorophyll and adaptation to a saprophytic existence. If this were the case, creeping motility would have developed at a comparatively early stage in the evolution of the *Myxophyta* rather than among the *Hormogonales*. Jahn's (1924) claim to have observed creeping motility in unicellular blue-green algae would, if substantiated, provide strong support for this hypothesis.

C. *The Spirochaetes*

Our knowledge of the spirochaetes as a general group is still regrettably scanty. They are unicellular, spiral organisms, often considerably elongated, which, like the myxobacteria, possess a degree of flexibility never found in the *Eubacteriales*. They are motile either by means of an elastic axial filament or a modified fibrillar membrane. Division is always by means of transverse fission.

Their relationships are obscure. It has at times been suggested that they are related to the protozoa; however, the absence of true nuclei argues against this. On the other hand, their peculiar and specialized method of locomotion separates them sharply both from the true bacteria and from the myxobacteria. At present it seems impossible to arrive even at a tentative hypothesis as to their phylogeny. This is a group on which both morphological and physiological studies are greatly needed.

D. Other organisms commonly placed among the bacteria

Although the spirochaetes are the last of the clearly recognizable major groups of bacteria, there are still a number of organisms not falling into any of the three groups so far discussed which have in the past been included in the *Schizomycetes*. These are chiefly the forms placed by Bergey *et al.* in the orders *Chlamydobacteriales* and *Caulobacteriales*, with the addition of the non-photosynthetic members of the *Thiobacteriales*.

The colorless representatives of the *Thiobacteriales* have been divided into two morphologically quite distinct families, the *Beggiatoaceae*, and the *Achromatiaceae*. The genera *Beggiatoa*, *Thiothrix*, and *Thioploca*, comprising the former of these families, possess a number of morphological characters (shape, size, method of locomotion, sheath formation) which make it possible to consider them as *Hormogonales* which have lost their pigment system. They represent the colorless counterparts of the blue-green genera *Oscillatoria*, *Phormidium*, and *Schizothrix*. This has long been recognized by botanists and microbiologists, such as Winogradsky (1888) and Pringsheim (1923).

The family *Achromatiaceae* contains organisms which so far have been studied exclusively from field collections. The genus *Thiospira*, whose representatives are morphologically typical spirilla, is so different from the other genera that there is every reason for its removal from the family and inclusion in the *Eubacteriales* in the family *Pseudomonadaceae*. The remaining genera are all very similar to one another morphologically, and it would seem wise to consider them as members of a single genus (*Achromatium*) pending more careful studies under controlled conditions. The relationships of these organisms are at present obscure. Their size and internal structure makes it unlikely that they are related to any of the three major groups of bacteria. Judging from the most reliable reports, (e.g., Nadson 1914) their method of locomotion appears to resemble that of the blue-green algae.[2] In this connection it is tempting to compare them

[2] Although West and Griffiths (1909) have claimed that *Hillhousia mirabilis* is equipped with flagella, no other workers with organisms of this type have ever corroborated it.

with such members of the *Myxophyta* as *Synechococcus aeruginosus*, to which they might well be related in the same manner as *Beggiatoa* is to *Oscillatoria*.

The order *Caulobacteriales*, created by Henrici (1935) and accepted by Bergey *et al.* in the most recent edition of the *Manual* (1939), comprises a heterogeneous collection of organisms whose sole unifying character is the possession of a stalk. The provisional nature of the order is illustrated by the following quotation from Henrici (1935):

> It may be questioned whether the grouping of all stalked bacteria into a separate order is a "natural" arrangement, i.e., whether these organisms are actually phylogenetically related. Concerning this, we do not have enough information to venture an opinion, *but the same is equally true of the other orders of bacteria as now defined* (our italics).

The genera *Nevskia*, *Gallionella*, and *Caulobacter* appear to comprise organisms which are morphologically members of the *Eubacteriales* on the basis of our definition of that order. The species *Nevskia pediculata* is probably an unstalked lactic acid bacterium identical with *Betabacterium vermiforme* (Ward) Mayer.[3]

Nevskia ramosa, on the other hand, as shown by the illustrations of both Famintzin and Henrici (1935), possesses true stalks which are characteristically dichotomously branched in a manner similar to those of *Gallionella*. Obviously pure culture studies on these organisms are needed, and the relationships are as yet problematical. Nevertheless, as far as one can judge from the present descriptions, *Nevskia ramosa* and the *Gallionella* species seem to have morphological characters in common which are not encountered in the other genera of Henrici's order *Caulobacteriales*.

The occurrence of longitudinal fission and budding in the members of the family *Pasteuriaceae* shows that these organisms at least differ markedly from the true bacteria. Even after the extensive morphological studies of Kingma Boltjes (1936) on pure cultures of one member of the family, *Hyphomicrobium vul-*

[3] Cf. in this connection Mayer's (1938) drawings of the capsules of *B. vermiforme*.

gare, it is impossible to arrive at any well-founded conclusion concerning the relationships of the group.

Of the organisms included in the order *Chlamydobacteriales*, *Clonothrix fusca* is probably a colorless blue-green alga whose counterpart might be found in the myxophytal genus *Rivularia*. The relationships of *Crenothrix polyspora* and of the genera *Leptothrix* and *Sphaerotilus* are not clear. The morphological characteristics of *Crenothrix* are so outstandingly different from those of the other two genera that a single family containing all of them seems indefensible.

6. THE SYSTEMATIC POSITION OF THE BACTERIA

The foregoing pages have, we feel, justified the claim that a phylogenetic approach to the taxonomic problems of the bacteria is capable of yielding fruitful results. It is true that there are a small number of organisms of whose relationships we are still ignorant, but if it be remembered that these are mostly microbes not as yet studied under laboratory conditions, it may be expected that further work will result in an elucidation of their taxonomic positions. All the other bacteria can be readily subdivided into three large groups whose sharp and easy separation on the basis of one or two fundamental morphological characters is possible.

It is more difficult to give a precise yet adequate definition covering all the organisms in these three groups. The morphological criteria that can be used are exclusively negative—*viz.*, the earlier mentioned absence of true nuclei, of sexual reproduction, and of plastids. But these alone are not enough to characterize the bacteria, since they apply equally well to the *Myxophyta*. Thus, we are forced to realize that on a morphological basis alone the separation of the bacteria and the blue-green algae is impossible. Whereas the higher groups of the *Myxophyta* (e.g., the *Beggiatoaceae*) can be adequately distinguished from the bacteria proper on a strictly morphological basis, we must face the fact that so far as the *Chroococcales* are concerned this is not so.

Hence, if the *Myxophyta* are to be retained in their present form as a division, and if a purely morphological definition of the bacteria fails to keep out the members of the blue-green algae, the obvious conclusion would seem to be the creation of a kingdom in which the *Myxophyta* and the bacteria constitute the two at present recognizable divisions. Other taxonomists (e.g., Smith 1933, Copeland 1938) have been previously forced to the same conclusion. Copeland has pointed out that the name *Monera*, proposed by Haeckel, should be used for this kingdom in preference to *Schizophyta*—the name given by Cohn to the combined groups of blue-green algae and bacteria. Since Copeland's arguments seem sound, the name *Monera* will be adopted here.

A subdivision of the kingdom *Monera* can be achieved along at least two rather different lines, each one recognizing certain definite relationships.

The first method would be a splitting into three divisions on the basis of the method of locomotion. These three divisions would be: firstly, the *Myxophyta* in the usual sense, with the addition of the *Myxobacteriales* and the *Beggiatoaceae*, i.e., all organisms which, if motile, show creeping motility; secondly, the *Eubacteriales*, the *Actinomycetales* and the *Rhodobacteriales*, which, if motile, possess flagella; and thirdly, the spirochaetes, which are motile by means of an axial filament or a fibrillar membrane. Whereas this scheme accords most closely with our concept of the phylogeny of the *Monera* (see fig. 2), it has at present one grave disadvantage. This is the allocation of non-motile organisms. Since permanent immotility is so common in the first two groups, we feel that this scheme would be at present unworkable. It has been mentioned nevertheless because future work may lead to the discovery of one or more correlating morphological characters which would obviate the arbitrary assignment of a non-motile species to either one of the first two units.

The second possibility is suggested by the desirability of maintaining the *Myxophyta* as an independent unit. This implies that the subdivision of the *Monera* must be based on physiological characters, a subdivision all the more difficult because the designation "photosynthetic" for the *Myxophyta* has to be qualified.

The basic reason for this unfortunate predicament is the fact, mentioned previously, that the only possible consistent basis for distinguishing between the *Eubacteriales* and the *Chroococcales*—both of them key groups which it is obviously desirable to keep apart, since they form the starting points for two major evolutionary lines—is physiological.

To make this point perfectly clear the consequences of the two alternative methods of subdivision will be elaborated. In the former, an immotile organism, either belonging to the *Myxophyta* or to the *Eubacteriales*, but which is not clearly related to motile organisms in one of these two groups, is in a doubtful position.

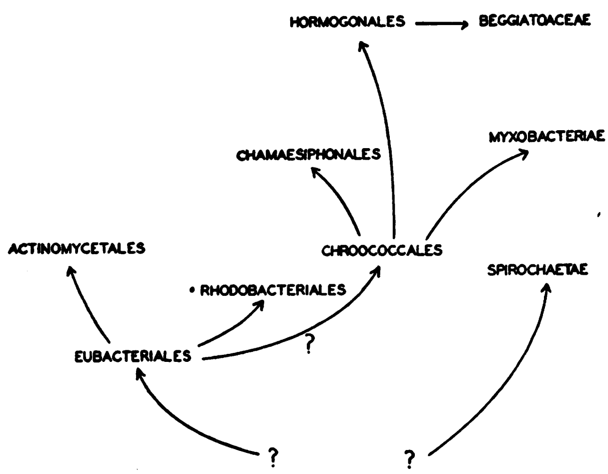

FIG. 2. INTERRELATIONSHIPS OF THE *Monera*

Yet a taxonomist would not have the least hesitation in placing a typical (immotile!) blue-green *Chroococcus* in the first group, an action which would imply that he had made use of the photosynthetic mechanism in determining its position. This shows that in doubtful cases (i.e., all immotile forms!) for the ultimate determination of systematic position physiological characters would have to be used.

In the latter case, it is the position of all colorless, non-photosynthetic members of the *Myxophyta* which is uncertain. By recognizing that in some cases morphological criteria *alone* can satisfactorily place an organism in the *Myxophyta*, we can make possible the inclusion of such forms as *Beggiatoa* here.

But we must resign ourselves to the fact that the colorless representatives of the larger part of the *Chroococcales* cannot be so treated and will probably be placed among the bacteria. In such cases the question whether a colorless organism shall be considered to belong to the bacteria or to the *Myxophyta* will, at least for the present, remain a matter of scientific tact. In order to forestall possible criticism on this matter, we should like to point out that exactly the same difficulties, although perhaps not so clearly apparent, exist in all other systems of classification, including that of Bergey *et al.*

For practical reasons, then, we propose the adoption of a subdivision of the kingdom *Monera* into a division of blue-green algae (*Myxophyta*) and a division of bacteria (*Schizomycetae*) in accordance with the second scheme outlined above. The definitions of the kingdom and its two divisions are as follows:

KINGDOM MONERA Haeckel.

Microörganisms which do not possess true nuclei or plastids, and which do not exhibit sexual reproduction.

DIVISION I. MYXOPHYTA. Unicellular or multicellular organisms, which, if motile, show creeping motility. The predominant type of metabolism is photosynthetic with oxygen production. The photosynthetic pigments are chlorophylls a and b, accompanied by phycocyanin and sometimes phycoerythrin. Non-photosynthetic colorless organisms which are clearly recognizable as counterparts of photosynthetic genera are also included.

DIVISION II. SCHIZOMYCETAE. Unicellular or mycelial organisms, which, if motile, may creep or may move by means of flagella or an elastic axial filament or fibrillar membrane. Metabolism is predominantly non-photosynthetic, but if photosynthetic is without oxygen production. The photosynthetic members of the division never contain chlorophylls a or b, phycocyanin or phycoerythrin, and, if motile, are always flagellated.

7. OUTLINE FOR THE TAXONOMY OF THE SCHIZOMYCETAE

The further subdivision of the *Myxophyta* lies outside the scope of this paper, so we may confine ourselves to the more detailed taxonomy of the *Schizomycetae*.

For the reasons given previously, we believe that the three major groups recognized among the bacteria are of polyphyletic origin. Hence it seems both natural and desirable to give each the rank of a class. The following names and definitions are proposed:

CLASS I. EUBACTERIAE. Unicellular or mycelial organisms with rigid cell walls. If unicellular, they may be spherical, rodshaped or spiral. Motility, when present, always by means of flagella. Multiplication by transverse fission. Resting stages, if present, may be endospores, cysts or conidia.

CLASS II. MYXOBACTERIAE. Unicellular rod-shaped organisms, without rigid cell walls, which always show creeping motility (never flagella). Multiplication by transverse fission. Resting stages, if present, may be microcysts, sometimes contained within larger cysts. The individual microcysts or the larger cysts may be borne on or in fruiting bodies of various shapes.

CLASS III. SPIROCHAETAE. Unicellular spiral organisms without rigid cell walls, always motile by means either of an elastic axial filament or of a modified fibrillar membrane. Multiplication by transverse fission. No resting stages known.

The class *Eubacteriae* comprises the three natural assemblages of the true bacteria, the photosynthetic bacteria and the actinomycetes. Thus, a subdivision of the class into three orders, each representing one of these groups, would seem appropriate.

Nevertheless, this procedure is far from satisfactory. The orders so created are by no means equivalent, since the photosynthetic bacteria are only distinguishable from certain genera in the *Eubacteriales* by means of physiological criteria, while the *Actinomycetales* constitute a morphologically characterized unit. Furthermore, the latter order should logically include the genera *Mycobacterium*, *Corynebacterium* and *Propionibacterium*, as well as the rod-shaped and spherical lactic acid bacteria, all of which are clearly related to the actinomycetes proper. However, if we carry the *Actinomycetales* down into the *Eubacteriales* in this manner, it is only logical to recognize other morphological lines in the *Eubacteriales*, such as the polarly flagellated, gram-negative rods, vibrios and spirilla now for the most part collected in the family *Pseudomonadaceae*, as orders. In our opinion, this would be the most desirable method of subdividing the class *Eubacteriae*. However, as has been pointed out previously (section 5A), the recognition of the necessary all-inclusive, homogeneous and equivalent groups within the *Eubacteriales* is not at present possible. Particularly the affinities of the motile, gram-positive organisms and of the peritrichous gram-negative bacteria are still obscure. As a consequence of this, it is very difficult to define unambiguously even such groups as the *Actinomycetales*. If non-mycelial organisms are to be included in this order, the definition must rest largely on the outcome of the gram stain. In view of the motile streptococci and the motile *Mycobacterium*- or *Proactinomyces*-like organisms described by Topping, it is no longer feasible to add the further designation "permanently immotile", the only one which would ensure a separation from the spore-forming bacteria and from such genera as *Kurthia* and *Listeria*. With an increase in understanding of the relationships of the motile gram-positive and peritrichous gram-negative types, such difficulties may well disappear and the logical subdivision hinted at above may become possible. Until that time, we deem it best to retain the long recognized taxonomic entities in the *Eubacteriae*—the true bacteria, the photosynthetic bacteria and the actinomycetes—and to set them up as orders. For the sake of clear definition, the order *Actinomycetales* must, unfortunately, be confined to the mycelium-forming organisms.[4] Pringsheim's order *Rhodobacteriales* will be used to accommodate the photosynthetic bacteria.

The definitions of the three orders are as follows:

ORDER I. RHODOBACTERIALES Pringsheim *emend.* Unicellular organisms. No resting stages known. Photosynthetic, not producing oxygen.

[4] In a personal communication Dr. H. L. Jensen recently proposed the delimitation of the order *Actinomycetales* on the basis of conidia formation rather than the presence of a mycelium. This would obviate the previously stressed difficulty inherent in the separation of the genera *Mycobacterium* and *Proactinomyces*. Therefore we feel that Jensen's proposal should be adopted. It will necessitate only minor changes in the definitions and key. (Note added during the correction of proof).

ORDER II. EUBACTERIALES Buchanan *emend.* Unicellular organisms. Resting stages, if present, may be either endospores or cysts. Non-photosynthetic.

ORDER III. ACTINOMYCETALES Buchanan *emend.* Mycelial organisms. Resting stages conidia; never endospores or cysts. Non-photosynthetic.

The order *Rhodobacteriales* includes the sulfur purple bacteria, the non-sulfur purple bacteria, and the green bacteria. The order *Eubacteriales* includes, in addition to those organisms placed here by Bergey *et al.*, the genera *Mycobacterium, Corynebacterium, Erysipelothrix,* and *Leptotrichia,* which are placed by Bergey in the order *Actinomycetales;* the genera *Nevskia, Gallionella,* and *Caulobacter*, placed by Bergey in the order *Caulobacteriales;* the genus *Thiospira,* placed by Bergey in the order *Thiobacteriales;* and the genera *Siderocapsa* Molisch and *Sideromonas* Cholodny which are not at present included in the *Manual.* The order *Actinomycetales* includes those groups recognized by Waksman (1940) with the exception of the *Mycobacteriaceae.*

At present, one order seems quite sufficient for the class *Myxobacteriae.* This is the order *Myxobacteriales* Jahn, with definition as for class. It should be mentioned, however, that we include in this class the genus *Sporocytophaga* and the family *Cytophagaceae* (Stanier 1940), whose members were placed among the *Spirochaetales* in the latest edition of Bergey's *Manual.*

In the class *Spirochaetae* also one order seems sufficient. This is the order *Spirochaetales* Buchanan, with definition as for class.

We have still to consider the problem of dealing with those organisms ordinarily classed with the bacteria which do not fall into any of the groups discussed so far. The *Beggiatoaceae* and probably the genus *Clonothrix* belong, on the basis of the definition given above, in the division *Myxophyta.*

The remaining forms which have been incompletely studied, or which possess characters not permitting of their inclusion in either of the two divisions as now defined, are here collected in a provisional appendix. This procedure has also been suggested by Henrici (personal communication).

The following very tentative outline of this appendix is proposed:

Appendix to the division *Schizomycetae*.

Group I. Filamentous, ensheathed organisms.

Family 1. *Leptotrichaceae* Hansgirg. Unbranched filaments of even width, attached or free. Multiplication by flagellated swarmers produced by division in one plane only.

Genera *Leptothrix* and *Sphaerotilus*.

Family 2. *Crenotrichaceae* Hansgirg. Unbranched filaments, tapering from the tip to the base, attached. Multiplication by non-motile conidia, produced by division in three planes.

Genus *Crenothrix*.

Group II. Unicellular organisms, multiplying by transverse fission.

Family 1. *Achromatiaceae* Buchanan *emend.* Very large oval to spherical cells, with central vacuoles (?), containing sulfur globules and-or "oxalites" (?). Cell division by constriction. Motility similar to that of the blue-green algae (?), never flagellar.

Genus *Achromatium*.

Group III. Unicellular organisms, multiplying by longitudinal fission or budding or both.

Family 1. *Pasteuriaceae* Laurent *emend.* Henrici and Johnson. Stalked bacteria with spherical or pear-shaped cells; if cells are elongated, long axis of cell coincides with axis of stalk.

Genera *Pasteuria*, *Hyphomicrobium* and *Blastocaulis*.

It will be clear that the system outlined above has a number of advantages over those previously proposed. The most conspicuous improvement appears to be that the new systematic arrangement has made it possible to use consistently the major morphological criteria in such a manner that they become mutually exclusive. Thus the systematic position of an organism within the division *Schizomycetae* is no longer subject to that uncertainty which is such a grave disadvantage of the system at present used in Bergey's *Manual.* As a result the new outline promises to be considerably more useful from a strictly determinative standpoint.

A second advantage is the simplification brought about by the recognition of larger taxonomic units (i.e., classes) for various of the groups. Instead of having seven rather amorphous and ill-defined orders, three new classes have been recognized and more rigorously defined. This obviates the objection raised by Breed, Murray and Hitchens (1940) that the seven orders into which the bacteria had been subdivided were too many. When the number of orders in a given class becomes unwieldily large it is obviously better to increase the number of classes rather than to attempt the difficult task of reducing the number of orders by combining two or more, which was the policy advocated by these workers.

Correlated with these two advantages is a third one, *viz.*, the closer approximation to a system expressing natural relations. The multiplicity of classes reflects better the postulated polyphyletic origins of the bacteria. A corollary of this is the fact that classes and orders as here proposed are more nearly equivalent than in Bergey's system.

One of the imperfections of this, as of all tentative natural systems, is the impossibility of assigning a definite systematic position to organisms whose relationships are uncertain. This has necessitated in the present case the organization of an appendix. In our opinion this is better and certainly more conducive to further research than the policy of forcing such organisms into a framework originally not designed to receive them, a policy which has the disadvantage of discouraging research through its suggestion of finality. One might regard this as a temporary imperfection which will tend to disappear as a result of further work.

An intrinsic difficulty of the new system is the separation of the two divisions. However, as has been pointed out previously, this is a difficulty inherent in all systems of bacterial classification, even though not always clearly recognized.

It should be emphasized that the system proposed does not by any means pretend to be a completed project; it is rather to be regarded as a first draft of a rational system whose details will be filled in and whose outline will be corrected by subsequent work. It is our hope that it will be so considered.

SUMMARY

Following a discussion of the problems involved in the creation of the larger taxonomic units among the bacteria, an outline has been presented for a more rational approach.

The final arrangement proposed can best be presented in the form of the following Key to the divisions, classes, and orders of the kingdom *Monera*, composed of microörganisms without true nuclei, plastids, and sexual reproduction.

A. Organisms photosynthetic with the evolution of oxygen and possessing the typical green plant chlorophylls, phycocyanin and sometimes phycoerythrin, and colorless, non-photosynthetic counterparts, clearly recognizable as such.
Division I *Myxophyta.*

B. Organisms not so characterized.
Division II *Schizomycetae.*

- I. Unicellular or mycelial organisms with rigid cell walls. Motility, when present, by means of flagella. Endospores, cysts, or conidia may be formed.
Class 1 *Eubacteriae.*
  - a. Organisms photosynthetic, but not producing oxygen.
  Order 1. *Rhodobacteriales.*
  - b. Non-photosynthetic organisms.
    - 1. Unicellular.
    Order 2. *Eubacteriales.*
    - 2. Mycelial organisms.
    Order 3. *Actinomycetales.*
- II. Unicellular rod-shaped organisms, without rigid cell walls. Always creeping motility. Microcysts and fruiting bodies may be formed.
Class 2. *Myxobacteriae.*
One order *Myxobacteriales.*
- III. Unicellular, spiral organisms without rigid cell walls. Motility by means of an elastic axial filament or modified fibrillar membrane.
Class 3. *Spirochaetae.*
One order *Spirochaetales.*
- IV. For organisms not falling into the previous classes see Appendix in text.

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